INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/CA 99/00933

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NICHOLS R ET AL: "A UNIVERSAL NUCLEOSIDE FOR USE AT AMBIGOUS SITES IN DNA PRIMERS" NATURE,GB,MACMILLAN JOURNALS LTD. LONDON, vol. 369, no. 6480, 9 June 1994 (1994-06-09), pages 492-493, XP000560346 ISSN: 0028-0836 cited in the application the whole document --- -/--	1-4, 16-25, 29-37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 2000	11/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Molina Galan, E

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/CA 99/00933

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	BONALDO FATIMA DE M ET AL: "NORMALIZATION AND SUBTRACTION: TWO APPROACHES TO FACILITATE GENE DISCOVERY" GENOME RESEARCH,US,COLD SPRING HARBOR LABORATORY PRESS, vol. 6, no. 9, 1 September 1996 (1996-09-01), pages 791-806, XP002039972 ISSN: 1088-9051 cited in the application page 798, last paragraph ---	1-37
Y	GUO, ZHEN ET AL: "Enhanced discrimination of single nucleotide polymorphisms by artificial mismatch hybridization." NATURE BIOTECHNOLOGY, (1997) VOL. 15, NO. 4, PP. 331 - 335. , XP000867755 cited in the application the whole document ---	1-37
Y	WO 97 18325 A (DAKO AS) 22 May 1997 (1997-05-22) the whole document ---	1-37
A	WO 94 06810 A (BERGSTROM DONALD EUGENE ;ANDREWS PHILIP CHARLES (US); NICHOLS RUTH) 31 March 1994 (1994-03-31) cited in the application ---	
A	LOH E Y ET AL: "POLYMERASE CHAIN REACTION WITH SINGLE-SIDED SPECIFICITY: ANALYSIS OF T CELL RECEPTOR DELTA CHAIN" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 243, no. 4888, 13 January 1989 (1989-01-13), pages 217-220, XP000673517 ISSN: 0036-8075 cited in the application -----	

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ional Application No
PCT/CA 99/00933

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9718325 A	22-05-1997	AU 7210196 A	05-06-1997
		EP 0862650 A	09-09-1998
		US 5888733 A	30-03-1999
WO 9406810 A	31-03-1994	US 5438131 A	01-08-1995
		CA 2144334 A	31-03-1994
		EP 0660842 A	05-07-1995
		JP 8501308 T	13-02-1996
		US 5681947 A	28-10-1997

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/08391

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C07H 5/04, 5/06, 19/00, 21/00, 19/06
US CL :536/18.7, 22.1, 26.1, 26.9, 28.6, 28.7, 28.8, 28.9
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/18.7, 22.1, 26.1, 26.9, 28.6, 28.7, 28.8, 28.9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Nucleosides and Nucleotides, Volume 7, Number 3, issued 1988, Ramasamy et al, "Synthesis of Brunfelsamidine Ribonucleoside and Certain Related Compounds By the Stereospecific Sodium Salt Glycosylation Procedure", pages 385-392, see entire document.	1-15
Y	Nucleic Acids Research, Volume 18, number 5, issued 1990, Dawson et al, "Syhthesis and Characterization of a Ribavirin-3',5'-Phosphate Pentadecamer Homoribopolymer Bearing a 5'-amino Tether Group and a 3'-thymidine", pages 1099-1102, see entire document.	16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 October 1993	Date of mailing of the international search report NOV 02 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer L. LANKFORD Telephone No. (703) 308-0196

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December 5, 2000

BY FACSIMILE AND BY MESSENGER

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European Patent Office
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Object: International Application No. PCT/CA99/00933
International Filing Date: June 10, 1999
Applicant: McGILL UNIVERSITY et al.
Title: OLIGONUCLEOTIDE PRIMERS THAT DESTABILIZE NON-SPECIFIC DUPLEX FORMATION AND USES THEREOF

Dear Sir/Madam :

This is in response to the Written Opinion dated July 5, 2000 and to the Communications regarding extension of time limit dated October 10th and November 13th, 2000, bringing the deadline for responding to December 5, 2000.

The Examiner has based his/her Written Opinion on the following three documents:

A Nichols et al.: 1994, Nature, 369:492-493;
B Bonaldo et al., 1996, Genome Research 6:791-806; and
C Zhen Guo et al., 1997, Nature Biotechnol. 15:331-335.

Document A

A teaches a non-discriminatory base analogue, or universal base

> "which maximizes stacking while minimizing hydrogen-binding interactions without sterically disrupting **DNA duplex**. Oligonucleotide containing M [the universal nucleoside] at several sites were used as primers for **sequencing** and the **polymerase chain reaction**" [emphasis added].

Document A teaches a new base analogue which aims at solving problems that arise due to the degeneracy of the genetic code.

Document A does not teach or suggests the use of such a universal nucleoside in the formation of a nucleic acid duplex between two homopolymeric sequences. In addition, document A is limited to DNA:DNA duplex formation and does not teach or suggest RNA:DNA duplexes. As well known in the art, the formation of DNA:DNA duplexes follow significantly different kinetics of hybridization than that of DNA:RNA duplexes (see, for example, Casey and Davidson, 1997, Nucleic Acids, Res. 4:1539). In fact, S1 mapping conditions are chosen in order to

> "minimize the formation of DNA:DNA hybrids while promoting the formation of DNA:RNA hybrids". (Sambrook et al., 1989, Molecular Cloning – A Laboratory Manual, 2nd Edition, Cold Spring Harbour Press, p. 7.58.)

The Examiner is also referred to page 7.63 of the classic text book from Sambrook et al. which demonstrates clearly the significant differences between the calculated melting temperatures (T_m for DNA:DNA (-·-·-) and DNA:RNA (---) hybrids (Appendix A, enclosed herewith for the Examiner's convenience).

Finally, document A does not teach or suggest molecular biology's methods which are dependent on a RNA-dependent DNA polymerase which make use of a universal analog. Document A merely refers to DNA-dependent DNA polymerase. As well know in the art, the kinetics of polymerization between a DNA-dependent DNA polymerase and that of a RNA-dependent DNA polymerase is significantly different (e.g. substrate affinity...).

The Examiner alleges that

> "The oligonucleotide is a homopolymer comprising at least one nucleotide modification [is] also disclosed in document A (see abstract and Figure 2)."

The Applicant respectfully disagrees with the Examiner's contention, since no homopolymers and no destabilization of a modified oligo to a non-homopolymeric

target sequence are taught in document A (neither in the abstract nor in Figure 2). The Examiner is respectfully referred to the "Definitions" section of the instant invention wherein the definition of "homopolymeric sequences" can be found. The Applicant also respectfully disagrees with the contention of the Examiner concerning the subject-matter of claims 17 and 21 since as argued above, document A merely teaches the use of an oligonucleotide containing at least one universal base in a method restricted to DNA dependent DNA polymerase. In any event, the arguments concerning the lack of teaching or suggestion of homopolymers in document A also apply to subsections 2.3, 2.4 and 2.5 of the Written Opinion.

The Applicant stresses that hybridization kinetics are dependent on the sequences of the two hybridizing partners. This fact is very well-known in the art (see Sambrook et al. 1989, for example). Thus, whether the modification of a homopolymeric oligonucleotide could help to destabilize non-specific binding to non-homopolymeric target sequences was not known or suggested prior to the demonstration of the present invention.

Document B

Document B relates to methods to produce cDNA libraries which are normalized as well as substractive methods to obtain other types of cDNA libraries. The methods described are quite complex methods described from pages 801 to 805.

Document B neither teaches nor suggests the use of a universal base to destabilize non-specific duplex formation between a homopolymeric target sequence and a modified homopolymeric oligonucleotide. The Applicant further notes that the authors, at page 798, right column, state:

> "Because of the relatively permissive conditions used for synthesis of first strand cDNA, priming with a *NotI*-tag-$(dT)_{18}$ oligonucleotide **may occur not only at the poly(A) tail of the mRNAs but also at internal A-rich sites within the mRNAs (e.g., at Alu tails)**." [emphasis added]

Thus, the authors stress that the oligo dT priming suffers from the same problems that the instant invention solves. Of note, Document B does not teach or suggest any means to correct it. Of course, the use of universal oligos to destabilize non-specific duplex formations between a homopolymeric target sequence and a modified homopolymer are not taught or suggested.

In accordance with one embodiment, the present invention aims at reducing mispriming due to artifactual duplex formations. In view of the significant amount of mispriming which occurs during cDNA synthesis for example (or other molecular biological methods) a fact discussed in document B, the present invention corrects major hurdles in the field of molecular biology.

Document C

Document C teaches the use of universal base analogs in order to discriminate single nucleotide polymorphisms (SNPs) in DNA hybridization by means of artificial mismatches. Thus, document C once again merely teaches the use of universal analogs to modulate melting temperatures of DNA duplexes. In addition, Document C is not concerned with the destabilization of non-specific duplexes, between an oligo and a non-homopolymeric target sequence, as it relates to the destabilization of heteropolymeric sequences. Finally, Document C is only concerned with DNA:DNA duplexes and with the methods based thereon (and not the discrimination of DNA:RNA duplexes).

The Applicant respectfully disagrees with the Examiner's contention at subsection 3.2 relating to obviousness because, for example,

"C (see figure 1; and pages 331, 333 and 335)"

do not concern homopolymers or DNA:RNA duplexes and to the use of mismatches in a modified oligonucleotide containing a homopolymeric sequence to destabilize non-specific duplex formation between the modified homopolymeric sequence and a homopolymeric target region.

Clearly, A, B or C, independently or taken together, teach or even suggest the use of mismatches (by universal bases or otherwise) in order to destabilize non-specific duplex between homopolymeric sequences. Neither of the documents, alone or together, teach nor suggest that such a destabilization could overcome the artifactual mispriming events and mismatches often encountered in molecular biology methods. In addition, the cited documents merely provide the teachings that a universal oligonucleotide can be used to discriminate between DNA:DNA duplexes and no teaching or suggestion as to what such a modification could do on RNA:DNA duplexes is given.

The Applicant respectfully submits that the instant application is the first which demonstrates that mismatches can be designed in order to destabilize non-specific

duplex formations between homopolymeric sequences. In addition, prior to the present invention, there had been no suggestion or experimental evidence demonstrating that such mismatches could be used in the context of RNA:DNA duplex formation and methods dependent thereon.

Since the Applicant is invited to correct certain defects in the International application, please amend this application as follows:

IN THE CLAIMS:

Please replace the complete set of claims by the new set submitted herewith.

REMARKS

Claims 1-36 are submitted.

The new set of claims, which is now presented, is based on the original set thereof, amended in view of the Written Opinion and to better define the subject-matter of the present invention.

More specifically, claim 14 has been canceled since it was a duplicate of claim 13. Accordingly, claims 15-37 have been renumbered so as to become claims 14-36. The claims have been amended to more clearly define the destabilizing effect of a modification in a homopolymeric sequence of an oligo on its duplex formation with a non-homopolymeric target sequence. Such a language is amply supported by the disclosure, but specific support can be found, for example, at page 6, from lines 20 to 26. A copy of a compare document of the claims is enclosed herewith for the Examiner's convenience. The claims have also been amended in order to delete the term "*bona fide*" in view of subsection 3 in item VIII of the Written Opinion.

In view of the amendments to the claims and the arguments submitted above, it is respectfully submitted that the claims, which now more specifically relate to the destabilization between a modified homopolymeric region of an oligo and a non-homopolymeric sequence of a target nucleic acid, are novel and inventive over A, B, or C, alone or in combination. Furthermore, it is believed that subsections 1 and 2 of item VIII have been rendered moot by the amendments to the claims.

In view of the foregoing, the applicant respectfully submits that the claimed subject-matter constitutes novel and non-obvious subject-matter and respectfully requests a favorable reconsideration thereof, in light of the above information.

Respectfully submitted,

GOUDREAU GAGE DUBUC

Charles Goyer, Ph.D.

Gaétan Prince

CG/lr
Encls: New set of claims;
Compare set of claims; and
Appendix A.

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

J. Sambrook
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

E.F. Fritsch
GENETICS INSTITUTE

T. Maniatis
HARVARD UNIVERSITY

1890–1990 CSH CENTENNIAL

Cold Spring Harbor Laboratory Press
1989

Hybridization buffer

40 mM PIPES (pH 6.4)
1 mM EDTA (pH 8.0)
0.4 M NaCl
80% formamide

PIPES: Use the disodium salt of PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]) to prepare the buffer, and adjust the pH with 1 N HCl.

Formamide: Many batches of reagent-grade formamide are sufficiently pure to be used without further treatment. However, if any yellow color is present, the formamide should be deionized by adding Dowex XG8 mixed-bed resin and stirring on a magnetic stirrer for 1 hour and filtering twice through Whatman No. 1 paper. Deionized formamide should be stored in small aliquots under nitrogen at −70°C.

4. Close the lid of the tube tightly, and incubate the hybridization reaction in a water bath set at 85°C for 10 minutes to denature the nucleic acids.

5. Rapidly transfer the tube to a water bath set at the desired hybridization temperature. Do not allow the tube to cool below the hybridization temperature during transfer. The hybridization temperature, which depends on the G + C content of the DNA, is chosen so as to minimize the formation of DNA:DNA hybrids while allowing DNA:RNA hybrids to form. Figure 7.5 shows the approximate hybridization temperatures for DNAs of different G + C content (Dean 1987). It is advisable to carry out a series of preliminary experiments to find out the optimal hybridization conditions for the RNA being used.

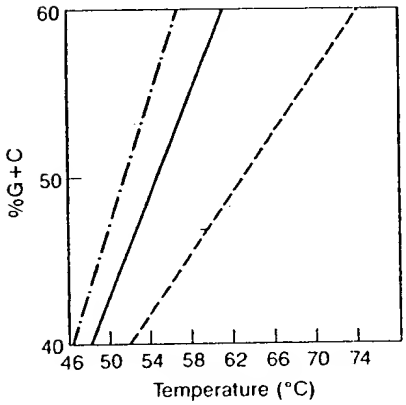

FIGURE 7.5
Optimization of yields of DNA:RNA hybrids. The graph (solid line) shows the temperature calculated to produce maximal yields of DNA:RNA hybrids when denatured DNA is annealed in the presence of RNA complementary to only one strand of the DNA. The broken lines show the calculated T_m for DNA:DNA (- · · · -) and DNA:RNA (---) hybrids. (Redrawn, with permission, from Dean 1987.)